PREPARATION AND CHARACTERIZATION OF INTERPENETRATING HYDROGEL OF HYDROXYETHYL METHACRYLATE AND METHYL CELLULOSE

A Thesis submitted in partial fulfilment of the requirements for the degree

Of

Bachelor of Technology
In

Biomedical Engineering

By

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Under the supervision of

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June, 2015

National Institute of Technology
Rourkela

CERTIFICATE

This is to certify that the thesis entitled, "***PREPARATION AND CHARACTERIZATION OF INTERPENETRATING HYDROGEL OF HYDROXYETHYL METHCRYLATE AND METHYL CELLULOSE***" submitted by **Mr. Abinash Lenka** in partial fulfillment of the requirements for the award of the degree of **Bachelor of Technology** degree in **Biomedical Engineering** at National Institute of Technology, Rourkela is an authentic work carried out by him under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other university/institute for the award of any degree.

Place : Rourkela

Date :

Supervisor

Dr. Sirsendu Sekhar Ray

Department of Biotechnology & Medical Engineering

NIT ROURKELA

ACKNOWLEDGEMENT

It is my pleasure to take the opportunity of expressing my sincere gratitude to all those people who provided their support, collaboration and encouragement to carry out my dissertation work. This training helps me a lot to extract out practical knowledge from theoretical work.

First of all I would like to thank my supervisor Dr. Sirsendu Sekhar Ray, Assistant Professor, Department of Biotechnology and Medical Engineering, NIT Rourkela for his invaluable guidance and help in my dissertation work. I also thank him for guiding me during every part of my work, for helping me improving upon my mistakes all through the project work and for his kind cooperation, inspiration and providing experimental expertise required in my work.

I also express my deep gratitude to Director, Dr. S.K .Sarangi, HOD, Professor (Mrs.)Krishna Pramanik, Dr. Kunal Pal and Dr. B P Nayak for providing me necessary research facilities.

I would like to thank to Ms. Priyanka Goyal, Ms. Alisha prsad, Ms. Sweta Naik , Mr. Rik Dhar, Mr. Narendra Babu, and Mr. Joseph Christakiranm ,Ms. Smaranika Panda for constant help during my work.

At last, I would like to express my sincere gratitude to my father, Mr. Kailash Chandra Lenka and my mother, Mrs. Ranjita Lenka for believing, inspiring and supporting me at every step of my life.

ABSTRACT

This thesis work is aims to give an overview of preparation and characterization of Interpenetrating hydrogels of hydroxyethyl methacrylate with methyl cellulose. Interpenetrating hydrogels were prepared by cross-linking the hydroxyethyl methacrylate and methyl cellulose in the presence of different concentration of ammoniumpersulphate and tetramethylethylenediamine. The concentrations of ammoniumpersulphate 20% (w/v), 40% (w/v) whereas the concentrations of tetramethylethylenediamine were 40% (v/v), 60% (v/v). The methyl Cellulose and hydroxyethyl methacrylate were mixed in the proportion of 30:70 (w/w), 20:80(w/w), and 10:90 (w/w) before being cross-linked using ammonium persulfate and tetramethylethylenediamine. At lower portion of Methyl cellulose hydrogels were oil type formulation. The hydrogels were characterized by swelling tests at pH 7, pH 1.2 and pH 7.4 and at temp 4^{o}c, where the swelling at pH 7 given the best result. Physical properties were characterized by XRD, FTIR and Mechanical study. In-vitro cytotoxicity was analyzed by Biocompatibility Test. The hydrogels were found to be biocompatible in nature.

Keywords: Interpenetrating hydrogels, Methyl cellulose, Hydroxyethyl methcrylate, Polymerization

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CHAPTER 1 : INTRODUCTION

1.1: Introduction

1.1.1 Hydrogels

Hydrogels are three dimensional (3D) polymeric systems that can hold vast measure of water inside it. They are super holding polymeric materials which have enormous parts in social protection especially for wound drug/ confirmation. This may be a result of their hydrophilicity, biocompatibility and biodegradability. From the human administrations purposes of perspective, hydrogel dressings have transformed into an extraordinarily interesting field of examination for the change of a simple to utilize restorative contraption for humankind. Different examination studies show that a moist injury environment is best for wounds to repair . The hydrogels holding both hydrophilic and hydrophobic areas on the macromolecular chains present amphiphilic (subsidiary) interesting properties, controlled by their hydrophilic/hydrophobic counterbalance. Hydrogels could be misleadingly relentless or reversible (physical gels) balanced out by nuclear catches, and in addition helper qualities including ionic, H-holding or hydrophobic associations, these hydrogels being non-homogeneous. Samples of reversible hydrogels are "ionotropic" hydrogels organized by the joint effort between a polyelectrolyte and an oppositely charged multivalent molecule, and the polyelectrolyte structures (complex coacervates) surrounded by the correspondence between two oppositely charged polyelectrolytes. Physical gels may be weakened by movements in nature's space conditions, for instance, ionic quality, ph, and temperature. Physical hydrogels have different biomedical procurements in pill movement, wound dressing, tissue outlining and so forth. Covalently cross-joined frameworks structure enduring or invention gels ""Smart"" hydrogels can basically change their volume/shape in light of little alterations of particular parameters of nature's turf. Responsive hydrogels have

different procurements, the lion's share of them being jogged around natural and healing demands, and detecting orders. Then again, single-framework hydrogels have frail mechanical properties and moderate response at swelling. Different systems from material science, microscale planning and microfluidics have been used to orchestrate biomimetic hydrogels.

1.1.2 IPN

These IPN hydrogels are composites of cross joined polymers, no less than one of them being coordinated and additionally cross-associated inside the snappy region of the other with no substance securities between them, which can't be separated unless the mixture securities are broken.There are different hydrogels concentrated around the polysaccharides and protein based IPN hydrogels. These interpenetrating polymer system hydrogels (IPN) have gotten a lot of thought in the late years, basically in perspective of their biomedical orders. They have procurements in medicine movement and separation frames, this is concentrated around the method for frameworks they have.

1.1.3 Poly (HEMA)

There has been a critical change in the field of 2-hydroxyethyl methacrylate (HEMA). HEMA is the monomer of the polymer poly (2-hydroxyethylmethacrylate). This polymer has the outstanding property to get swelled due to the molecule's hydrophilic pendant social affair when subjected to water. It is prepared for holding water from 10 to 600% in admiration to the dry weight. As an aftereffect of its uncommon property, it was one of the first materials to be used as a piece of contact lenses. Poly (2-hydroxyethyl methacrylate) orpoly (HEMA) is a champion amongst the most generally thought about hydrogels used as a piece of biomedical procurements. A thermoset that is not enzymatic associate undermined or hydrolysed by acidic arrangement. Different studies have been coordinated to modify poly (HEMA) with the purpose

of upgrading its mechanical properties. Its electro responsive properties expected to motivate better physiological responses.

1.1.4 Methyl cellulose

Cellulose is a direct homo-polymer polysaccharide having water insoluble property. Different cellulose subsidiary arranged by etherification increases water solvency and shows canteen responsive gelation. Thermo-responsive It is a semi-engineered polysaccharide and it contains hydrophobic spaces with in its polymeric structure. Methyl cellulose significantly investigated to plan temperature touchy bio therapeutic items. Watery arrangement of methyl cellulose have lower viscosities at low temperatures. Arrangements containing methyl cellulose or comparative cellulose subordinates are utilized as substitute for tears or spit if the common generation of these liquids is bothered. The methyl ether of cellulose, arranged from wood mash or cotton by treatment with antacid and methylation of the salt cellulose with methyl chloride. The article of business can be indicated further by thickness.

Methylcelluloses have an extensive variety of employments as a rule modern settings, contingent upon thickness (identified with atomic weight). They can be utilized for cements or thickening operators, thickness control specialists, or assurance in paint definitions. Pharmaceutical evaluations have been utilized as thickeners, covers, emulsifiers, and stabilizers in a mixture of corrective and sustenance items.

This thesis consists of preparation and characterization of two interpenetrating hydrogels used for various biomedical applications.In the IPNs HEMA is used as base material whereas HEMA and Methyl cellulose was added in varying concentrations before being cross-linked with APS and TEMED.

1.2. Objectives:

- To prepare the IPNs of Methyl cellulose and HEMA
- To characterize these IPNs by using different characterization technique

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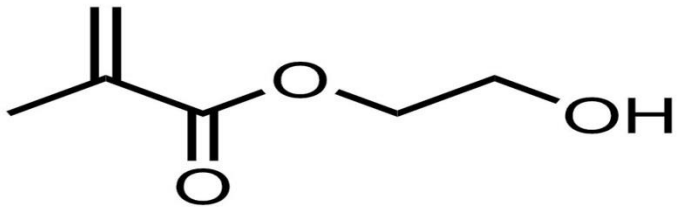
CHAPTER 2 : LITERATURE REVIEW

Hydroxyethylmethacrylate or HEMA is the monomer that is utilized to make the polymer poly(hydroxyethylmethacrylate). The polymer is hydrophobic; nonetheless, when the polymer is subjected to water it will swell because of the atom's hydrophilic pendant gathering. Contingent upon the physical and substance structure of the polymer, it is equipped for retaining from 10 to 600% water in respect to the dry weight. In light of this property, it was one of the first materials to be effectively utilized within the assembling of adaptable contact lenses.



(Hydroxyethyl)methacrylate

1.1(a)

Hydroxyethylmethacrylate or HEMA is the monomer that is used to make the polymer poly (hydroxyethylmethacrylate). The polymer is hydrophobic; in any case, when the polymer is subjected to water it will swell due to the molecule's hydrophilic pendant get-together. Dependent upon the physical and substance structure of the polymer, it is prepared for holding from 10 to 600% water in appreciation to the dry weight. In light of this property, it was one of the first materials to be adequately used inside of the amassing of versatile contact lenses.

Peppas, N.A., et al (2013) portrays the antifouling characteristics of the polysulfone cream ultrafiltration layer prepared with Tio2-g-HEMA. The polymeric material polysulfone (PSF) is by and large used inside of ultrafiltration layers as an aftereffect of its extraordinary mechanical

properties, strong manufactured tried and true qualities and wide pH operation range. Then again, the usage of polysulfone in prescription of water is restricted as a result of its hydrophobicity property which prompts genuine layer fouling and rot of permeability. Thus, it is imperative to change the PSF layer to improve its threatening to-fouling execution. Crossover layers surrounded by blending TiO2 nanoparticles and common materials are engaging for making new materials with enhanced properties, for instance, high permselectivity, incredible hydrophilicity and spectacular fouling wellbeing in a broad mixture of utilizations. In solicitation to enhance the hydrophilicity of movies and to thrashing the agglomeration of nanoparticles in movies and spillage of nanoparticles

Shah, D.K., et al., made a copolymeric bioadhesive skeleton with the likelihood to be utilized as a tissue glue revolved around biopolymer dextran. Copolymeric hydrogels including a urethane dextran (Dex-U) and 2-hydroxyethyl methacrylate (HEMA) were prepared and crosslinked under the bright (UV) light. Thickness of the outcomes and the cytotoxicity tests were dissected. Showed up distinctively in connection to Dex-U diagram, the augmentation of HEMA indispensably overhauled the properties of Dex-H structure particularly the hold quality and the nontoxicity. The copolymeric tissue glues shown guaranteeing handl quality and nontoxicity.

Liang, H.F., et al., describes the DNA-harm, cell-cycle capture and apoptosis actuated in BEAS-2B cells by 2-hydroxyethyl methacrylate (HEMA). The methacrylat monomer 2-hydroxyethyl methacrylate (HEMA) is usually utilized as a bit of gum-based dental therapeutic materials. These materials are cured in situ and HEMA and unmistakable monomers have been seen in including air all through dental surgery. In vitro studies have displayed an unsafe capacity of methacrylates, and concerns have been raised with respect to

conceivable wellbeing impacts because of interior breath. In this study, the frameworks of HEMA-influenced danger in the human lung epithelial cell line BEAS-2b. Consumption of cell glutathione (GSH) and an augmented level of delicate oxygen species (ROS) were seen after 2 h of presentation; however the levels were restored to control levels after 12 h. After 24 h, constrained cell duplication and apoptotic cell annihilation were found.

Aghaie et al. (2009) studies the adsorption elements of Cu2+ and Pb2+ particles onto Poly(HEMA) and (MMA-HEMA) surfaces from fluid singl solution. The adsorption benefits of Cu2+ and Pb2+ particles onto poly2-hydroxyethyl methacrylate (PHEMA) and copolymer 2-hydroxyethyl methacrylate with monomer methyl methacrylate adsorbent surfaces from watery single result were examined concerning the movements in the pH of result, adsorbent piece (changes in the weight rate of MMA copolymerized with HEMA monomer), contact time and the temperature in the diverse liquid results.

Shantanu Dhara et. al (2013) depicts the blend and portrayal of a hydrogel in light of dextrin joined with poly(2-hydroxyethyl methacrylate) by utilizing N,N-methylene bisacrylamide as cross linker, into a polymeric system in the presence of potassium persulphate (KPS) initiator for colon particular conveyance of ornidazole. Hydrogels [Dxt-g-p(HEMA)] arranged with distinctive fixation have been orchestrated by modifying the response parameters and the best one streamlined. The hydrogel was then described utilizing FTIR spectra, 13C NMR spectra, basic investigation, XRD study, SEM examination, TGA investigation, swelling study and cell suitability study. The harmony swelling proportion of the hydrogels has been inspected in diverse media and observed that it is greatest at pH 7.4. The investigation of cell practicality demonstrates that the hydrogel is non-cytotoxic in nature. The medication discharge system- and energy of ornidazole from

distinctive hydrogels have been researched utilizing direct and nonlinear numerical examination, which check that ornidazole discharge from hydrogel takes after first request energy and the component of non-Fickian dissemination.

CHAPTER 3 :MATERIALS & METHODS

3.1 Materials

The chemicals used:

Chemicals used and their formula:

Name of chemical	Chemical formula
Methyl Cellulose	R=CH3 (monomer)
HEMA(2-Hydroxyethyl Methacrylate)	$C_6H_{10}O_3$
APS(Ammonium Persulphate)	$(NH_4)_2S_2O_8$
TEMED(N,N,N,N-TeramethylEthylenediamine)	$C_6H_{16}N_7$

3.2 Methods

3.2.1 Preparation of Methyl cellulose

We have to make 40 ml of Methyl cellulose solution where methyl cellulose is 2%. First heat 20 ml of water using magnetic stirrer about temp of 50 c for half an hour. Then add 0.8 gm Methylcellulose powder and add another 20ml of water. Stir the solution using stir bar for half an hour.

3.2.2 Preparation of Methyl cellulose and HEMA IPNs

2ml of IPNs hydrogels of HEMA prepared with Methyl cellulose using different conc. of 1:9, 2:8 and 3:7 of Methyl cellulose and HEMA respectively. Add 33.3µl of APS(20/40) then shake it and the same amount (33.3µl) of TEMED solution (40/60)was mixed for crosslinking and for the formation of hydrogels they were kept in vacuum drier for 24 hours.

1.1(b)

Different tests and characterization technquies used for analyzing the properties of these interpenetrating hydrogels of Methyl Cellulose and HEMA are as follows :

- Swelling study
- Mechanical study
- X- Ray Diffraction (XRD)
- Fourier Transform Infrared Spectroscopy (FTIR)
- Biocompatibility

3.3 Swelling study.

The swelling study of methyl cellulose and HEMA hydrogel was carried out in three different pH scale:

a) At pH 1.2 buffer solution of HCl.

b) At pH 7 solution (distilled water)

c) At pH 7.4 buffer solution of ($Na_2HPO_{4)\ solution.}$

d) At distilled water at 4ºC

All these swelling studies were tested for 1 hour, 2hour, 3hour, 6hour, 12 hour, 24 hour and 48 hour in room temperature of 37° C. The hydrogels of each concentration of Methyl cellulose andHEMAwere made in triplets.

1.1(c)

3.3.1 Swelling study at pH 1.2 buffer solution of Hydrochloric acid (HCl) :

Initially 400ml of refined water was taken in a container, included 1 gm of sodium chloride and after that arrangement was mixed. Lastly. Six distinctive amassing of Methyl Cellulose and HEMA were taken in copies. The six diverse amassing of 0.1% w/v, 0.3% w/v, 0.2% w/v of Methyl cellulose and 0.9% w/v, 0.8%, 0.7%w/v of HEMA were taken. The Hydrogels of every fixation were made in triplets. The introductory weight of the hydrogels were taken and in the wake of drying it for 2 days in vacuum at 37oC their weight were measured for swelling study, then they were kept in the pH answer for 1hr, 2hr, 3hr, 6hr, 12hr, 24hr, 48hr thusly and their weights were measured. The mean and standard deviation of every specimens were taken out and plots was made between % of swelling mean versus time in hours.

3.3.2 Swelling study in pH 7 solution

This was done by keeping the samples of methyl cellulose and HEMA hydrogel of different concentration in double distilled water for the respective hours. The hydrogels of each conc. of methyl cellulose and HEMA were formed in 3X and their weights were measured is taken and again the plot was made between % of swelling mean versus time in hours.

3.3.3 Swelling study in pH 7.4 buffer solution of phosphate buffered saline(pbs)

The pH 7.4 cushion arrangement is like pH estimation. The hydrogels of every example of HEMA and methyl cellulose were framed in triplets. Swelling investigation of distinctive hydrogels of diverse fixation was performed in pH 7.4 support arrangement of Phosphate cushion arrangement .The mean and standard deviation of every examples were taken out and plots was made between % of swelling mean versus time in hours.

3.3.4 Swelling study of HEMA and MC solution at temp 4ºC in distilled water:

Swelling at 4ºC of HEMA and methyl cellulose in distilled water of six samples give the result of their capability of absorbing behavior. Samples are 1:9(20\40), 1:9(40\60),2:8(20\40),2:8(40\60), 3:7(20\40), 3:7(40\60). Swelling study of different hydrogels of different concentration was performed at 4ºC at distilled water. The mean and standard deviation of every samples were Calculated and plots was made between % of swelling mean vs time in hours**.**

3.4 X- Ray Diffraction:

The hydrogels of distinctive centralization of Methyl cellulose and HEMA were examined utilizing X-beam diffractometer. Cu – Kα radiation with wavelength 0.155 nm was utilized as a source. The machine was worked at 30 KV and 20 mA. Filtering of the specimens was done at 5° - 45°, 2θ with a rate of 5°2θ/min. The investigation was performed at the room temperature.

3.5 Fourier Transform Infrared Spectroscopy :

The readied hydrogels was inspected for spectroscopic examination utilizing FTIR spectroscopy ATR mode . These examples were investigated keeping air as the reference. Perusing of air is at first taken by the instument for foundation subtraction and afterward the specimens was put in instrument to record FTIR readings, hence subtracting the tops acquired via air. Filtering is done in the scope of 3000 cm-1 to 400 cm^{-1} with a determination of 5 $cm^{-1.}$

3.6 Mechanical Study:

The hydrogels are characterized by cyclic stretching process and repetitive stress relaxation. The hydrogels are made in thin film structure of 3mm length and 1mm width. These samples are tested under the 5mm/min stretching.

3.7 Biocompatibility:

The hydrogels of HEMA and MC prepared freshly. 100µl of sample taken in 96 well plates and it placed in UV radiation for 20 minutes for sterilization process. It firstly treated with 80% ethanol for removing toxicants and sterilize with PBS solution for removing ethanol unreacted monomer. The samples were washed with incomplete media DMEM low-glucose. Under 5% CO_2 atmospheric condition for their biocompatibility for 24 hours. The presence of viable cells are determined by MTT assay and OD process quantify the active cells.

CHAPTER 4 : RESULTS AND DISCUSSIONS

4.1 Gelation Time:

The Hydrogels formation time for Methyl cellulose was be half an hour and the time for Methyl cellulose and HEMA formation 24 hours keep it in 37ºc.

4.2 Swelling study:

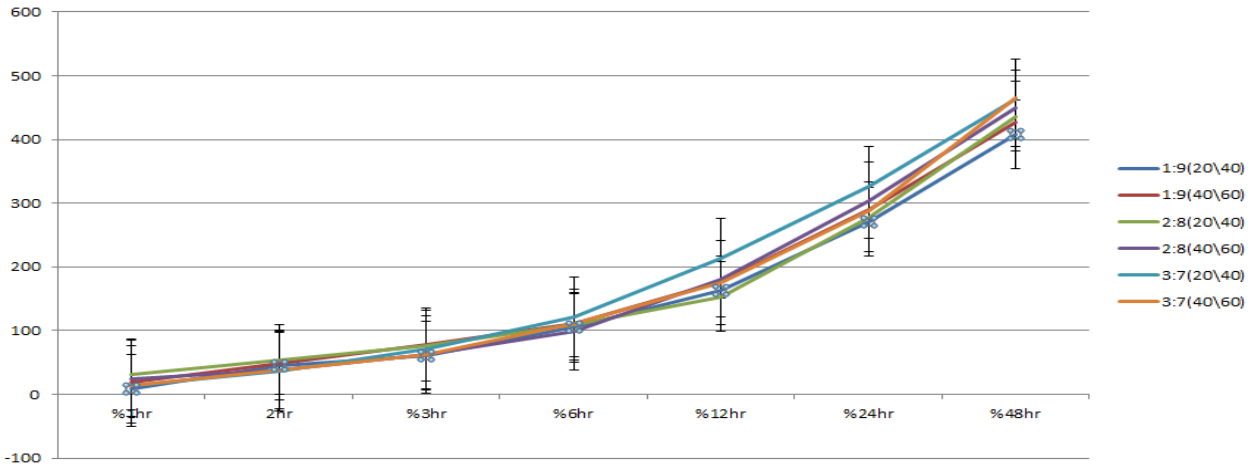
4.2.1 Methyl cellulose and HEMA hydrogel (at 37^{o}C)

$$\% \text{ Increase in Swelling} = W_f - W_i / W_i \times 100$$

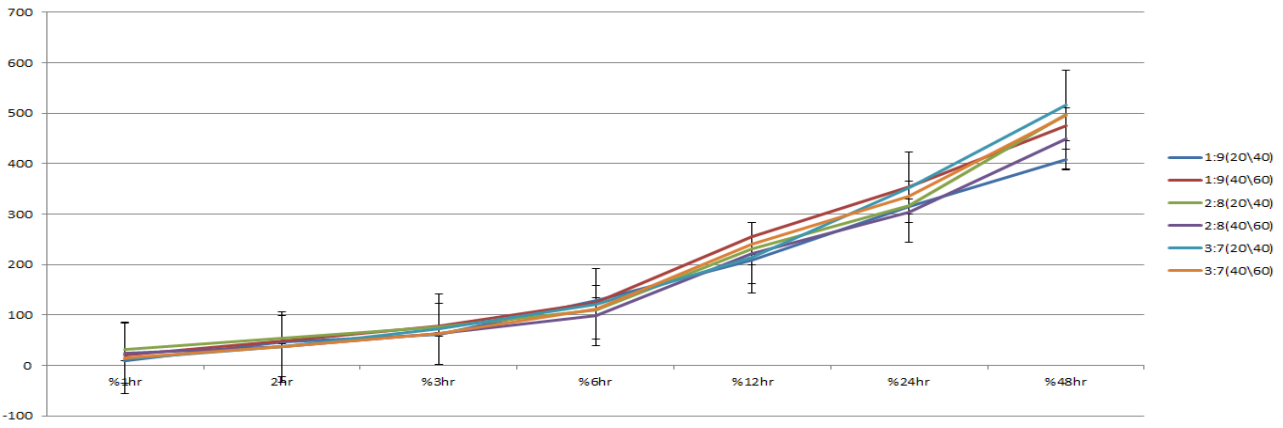
F_w : Final Weight of the Methyl cellulose and HEMA hydrogel sample

I_w : Initial Weight of the Methyl cellulose and HEMA hydrogel sample

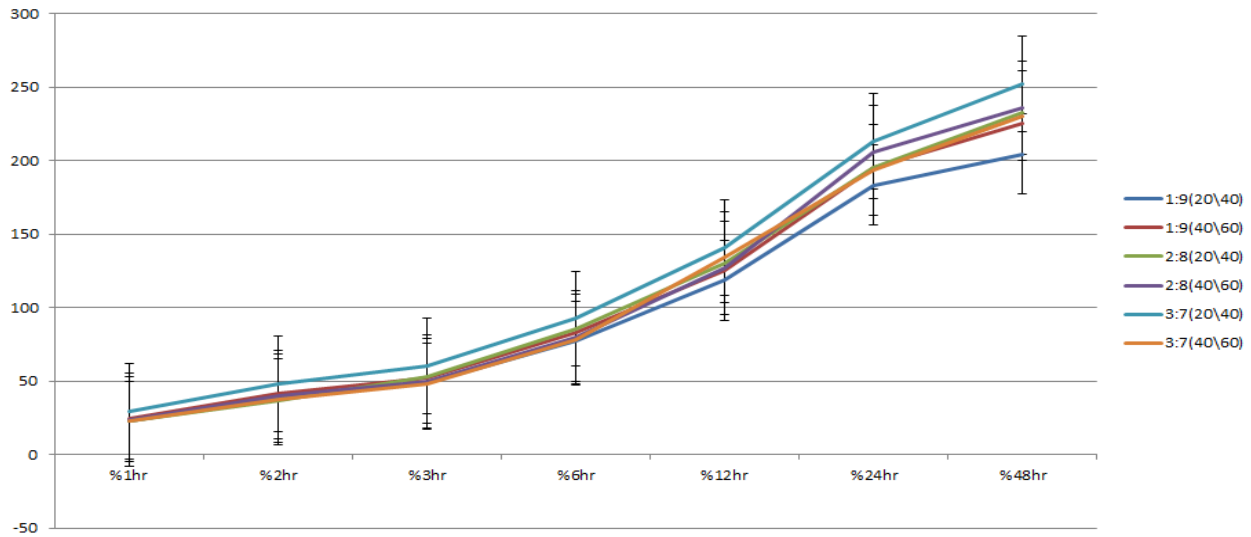
Swelling Study at PH 7:



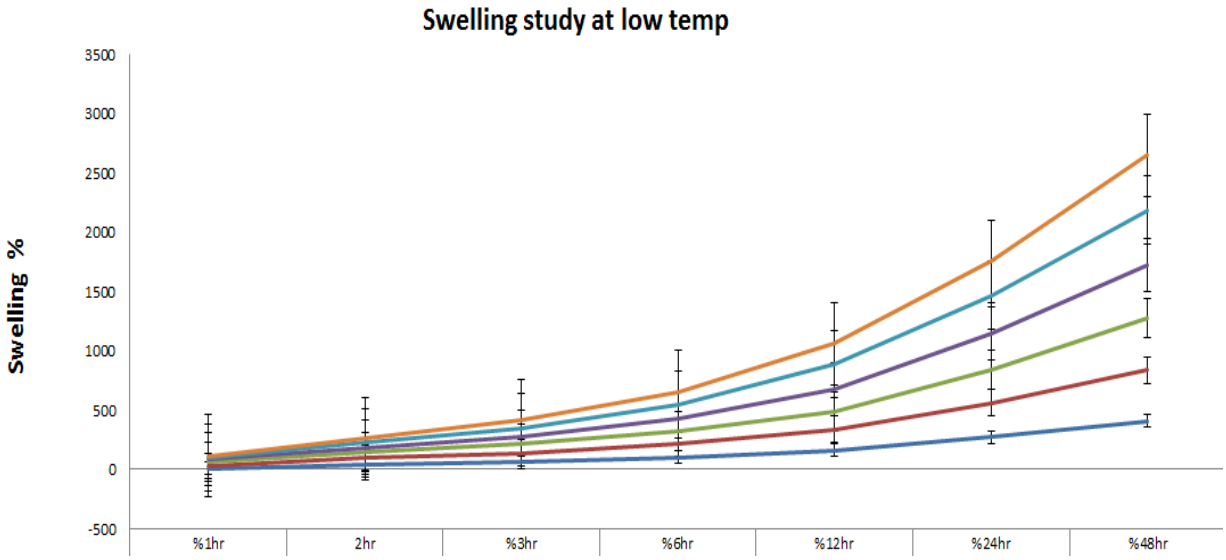
Swelling study at PH 1.2:



Swelling Study at PH 7.4:

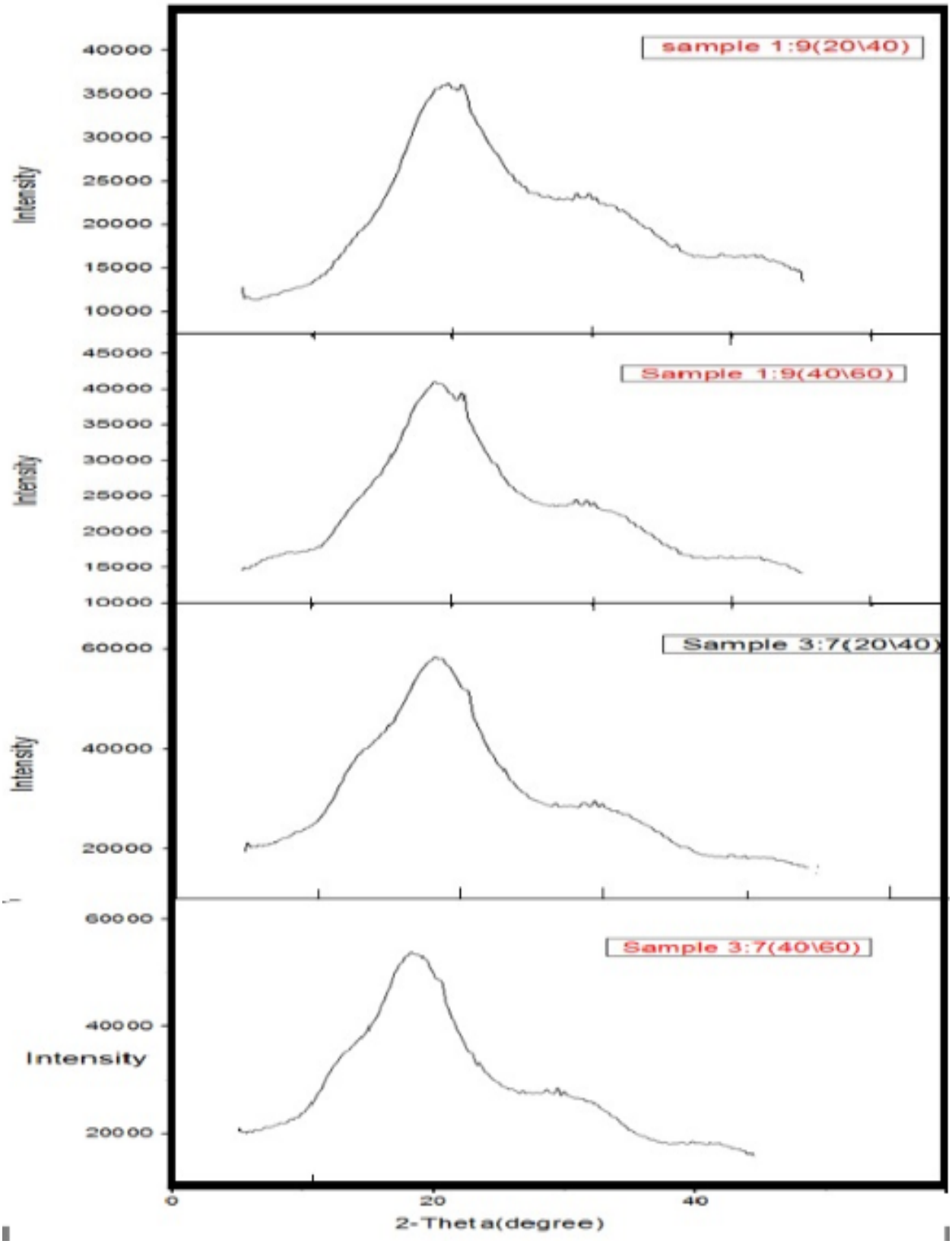


Swelling study of HEMA and Methyl cellulose at 4º c:



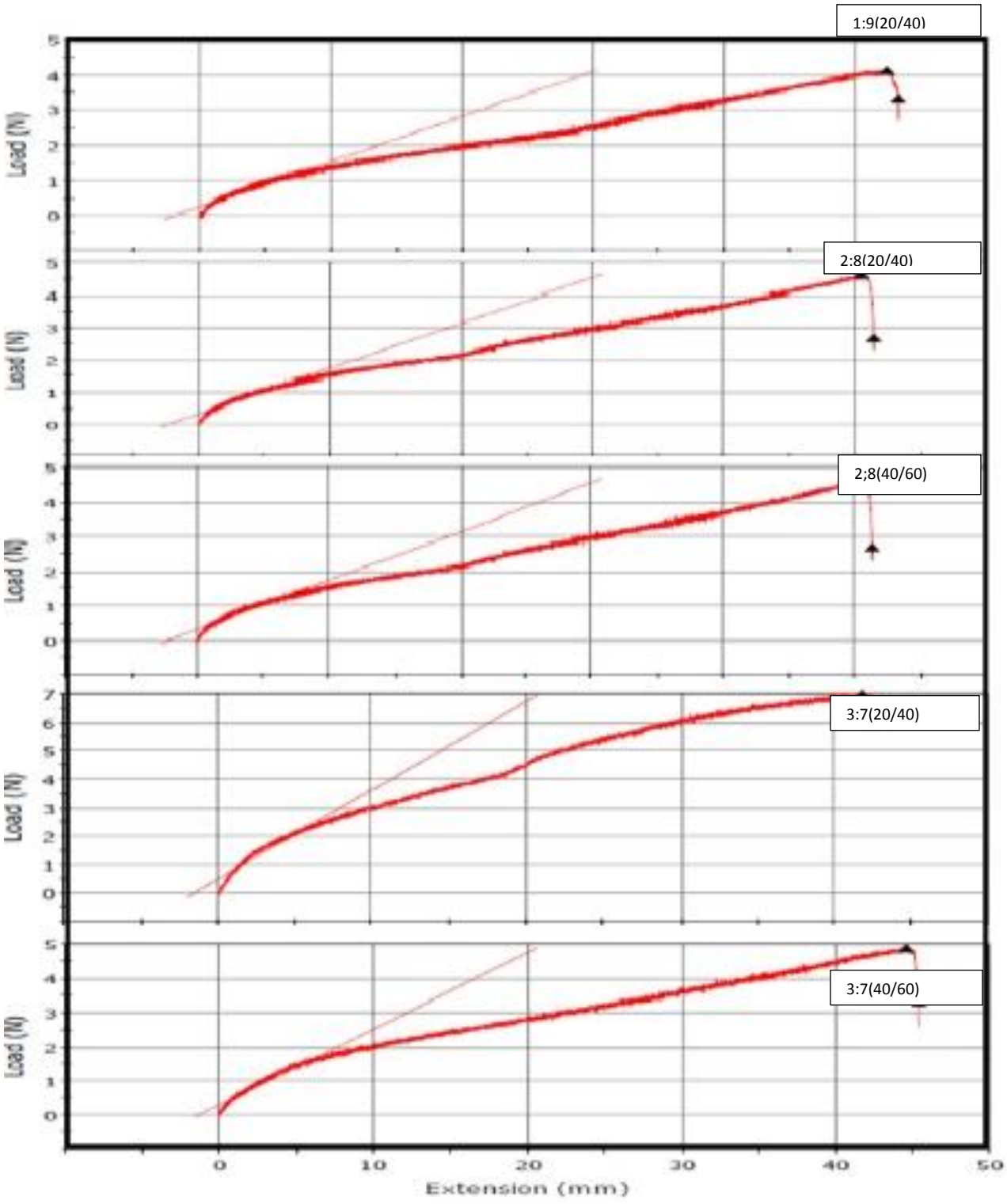
- Swelling effect in pH 1.2- sample 5> sample6~ sample3>sample2>sample4>sample 1
- Swelling effect in pH 7- sample 6>sample 5>sample 4>sample3>sample 2>sample 1
- Swelling effect in pH 7.4- sample5>sample 4>sample 6>sample 3>sample 2>sample 1
- Swelling effect at temp.4°C- sample 6>sample 5>sample 4>sample 3>sample 2>sample1
- So sample 6 30:70(40/60) gives the best result in every pH .

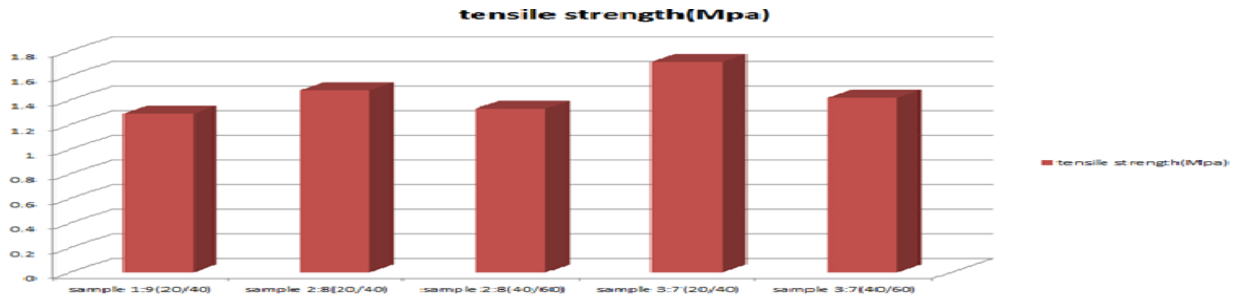
4.3X-Ray Diffraction:

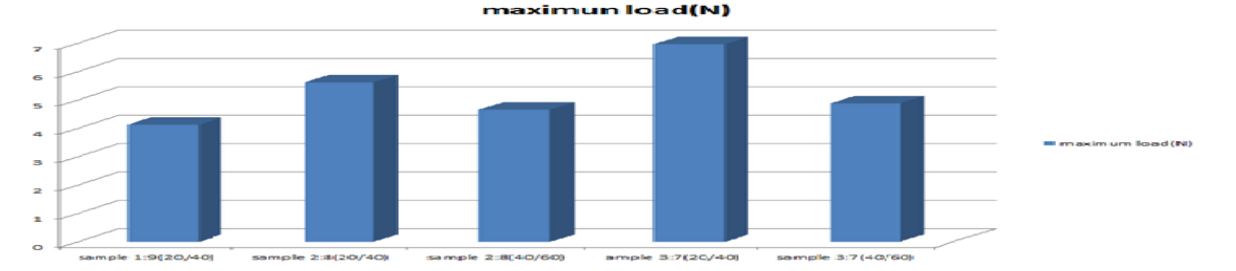


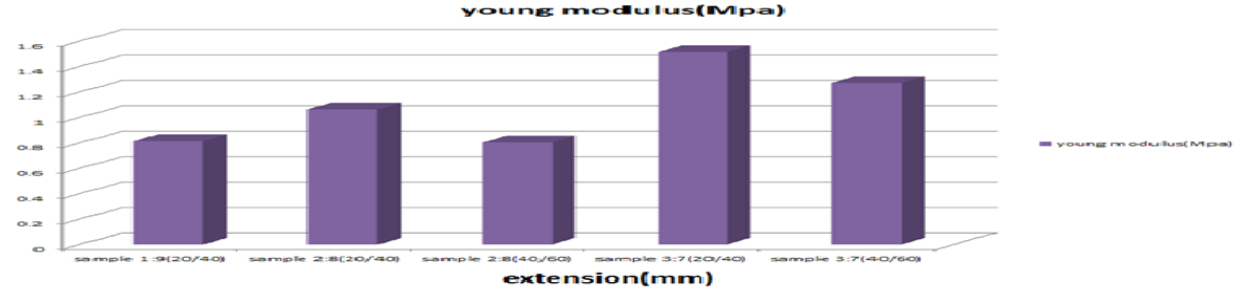
- XRD results gives crystalline behaviour of samples .
- There is peak in every samples at 19.5 ° ,which indicate the HEMA peak.
- Sample 1:9(20/40) gives picks when we going below the peaks are decreasing and gave smooth results.
- The peaks are because of presence of TEMED ,toxic in nature.
- 3:7(40/60) >3:7(20/40)> 1:9(40/60)> 1:9(20/40).
- So 3:7(40/60) sample give the best result among all the sample.

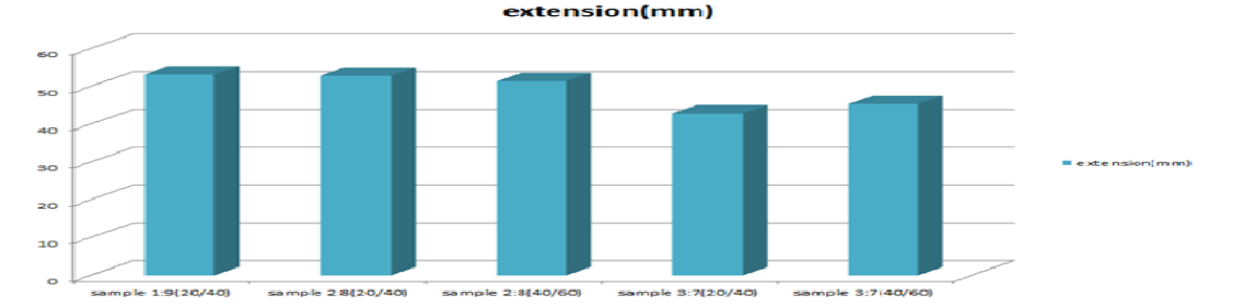
4.4 Mechanical study: (Tensile strength)

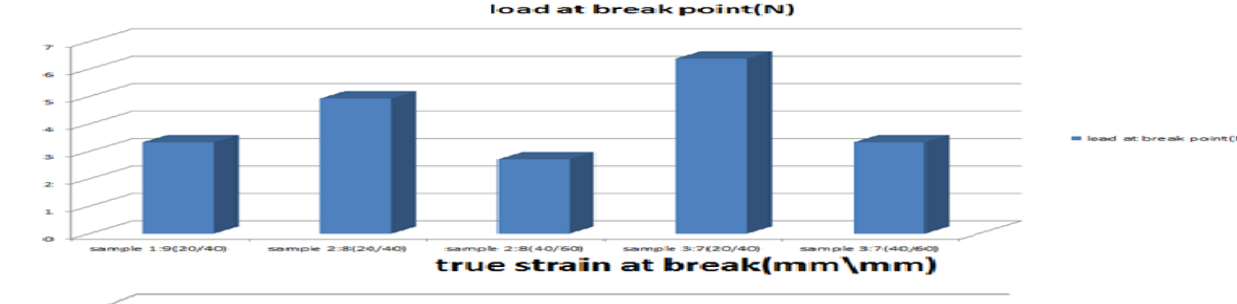


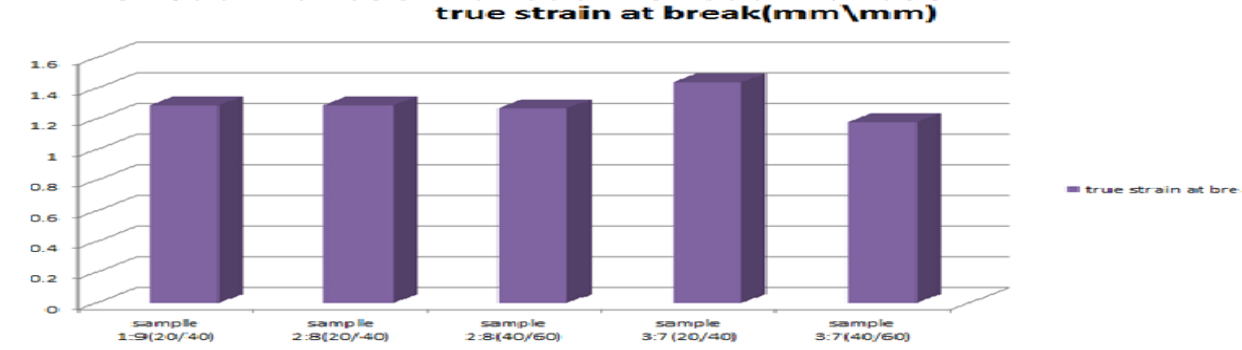

tensile strength(Mpa)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
sample 1:9(20/40)
sample 2:8(20/40)
sample 2:8(40/60)
sample 3:7(20/40)
sample 3:7(40/60)
tensile strength(Mpa)


maximun load(N)
7
6
5
4
3
2
1
0
sample 1:9(20/40)
sample 2:8(20/40)
sample 2:8(40/60)
sample 3:7(20/40)
sample 3:7(40/60)
maximun load(N)

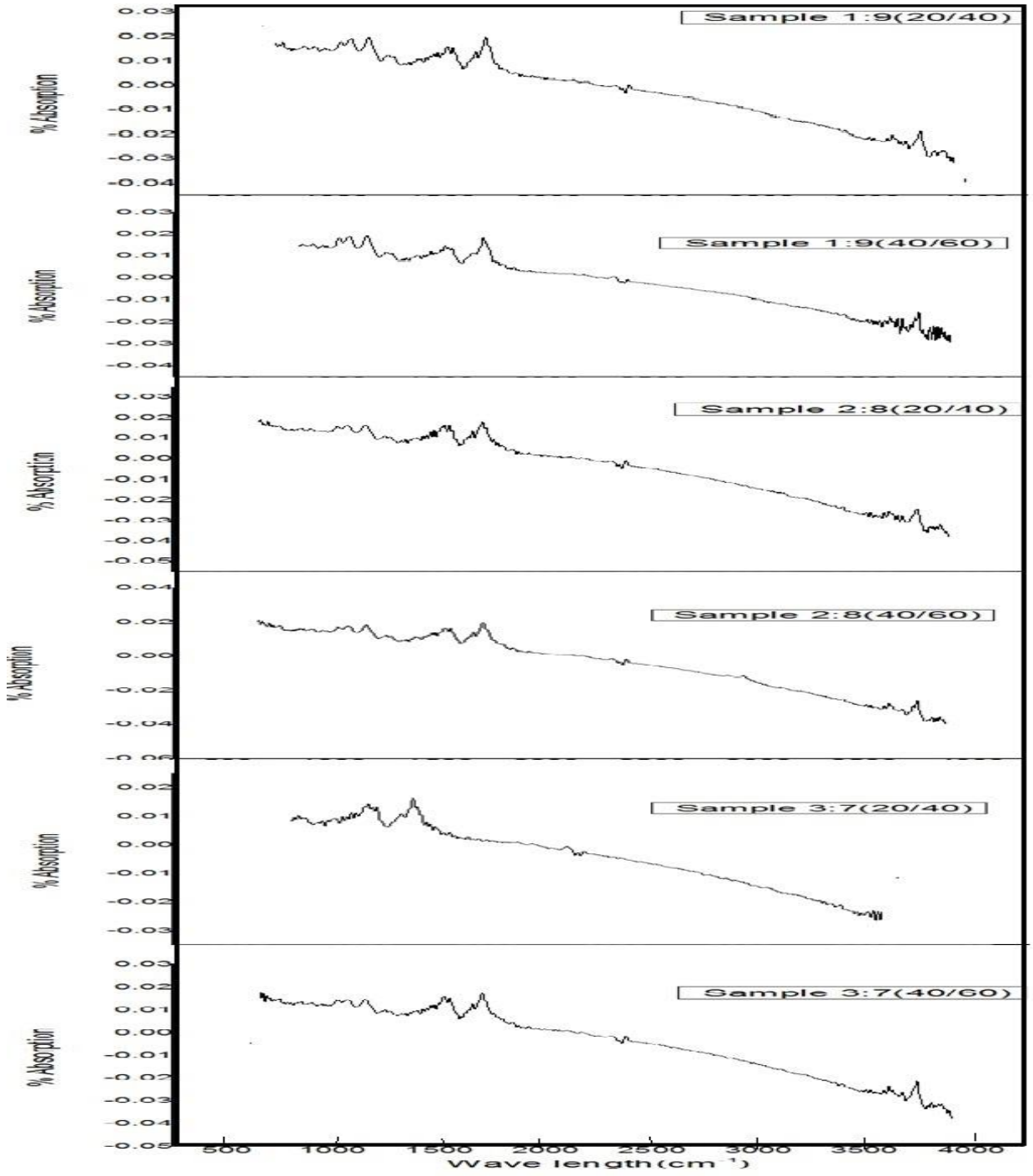

young modulus(Mpa)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
sample 1:9(20/40)
sample 2:8(20/40)
sample 2:8(40/60)
sample 3:7(20/40)
sample 3:7(40/60)
young modulus(Mpa)


extension(mm)
60
50
40
30
20
10
0
sample 1:9(20/40)
sample 2:8(20/40)
sample 2:8(40/60)
sample 3:7(20/40)
sample 3:7(40/60)
extension(mm)


load at break point(N)
7
6
5
4
3
2
1
0
sample 1:9(20/40)
sample 2:8(20/40)
sample 2:8(40/60)
sample 3:7(20/40)
sample 3:7(40/60)
load at break point(N


true strain at break(mm\mm)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
sample 1:9(20/40)
sample 2:8(20/40)
sample 2:8(40/60)
sample 3:7(20/40)
sample 3:7(40/60)
true strain at break(mm\mm

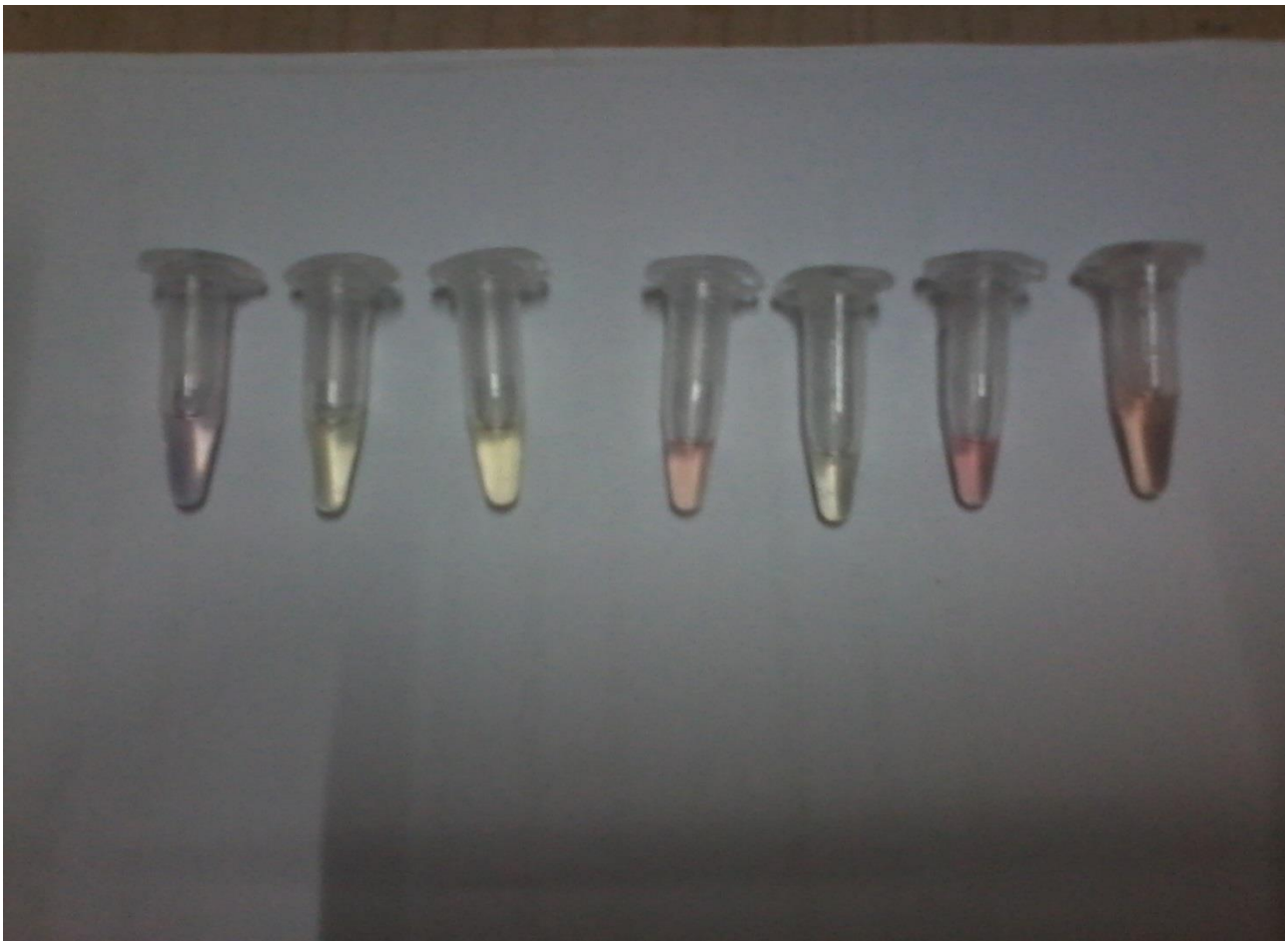
4.5 FTIR STUDY:



FTIR spectroscopy helps determine the functional groups occurring because of vibrations in between the bonds. From the study, it was observed that the wave number accounting to 3800 cm^{-1} is because of sinusoidal vibrations occurring because of environmental changes. Peak at 2500 cm^{-1} shows the C-N stretch for TEMED, which is evident in all the samples. The groove at 1550 cm^{-1} is indicative of methyl cellulose. The stretch at 1850 cm^{-1} shows the bonding between HEMA and methyl cellulose. Thus, from the FTIR analysis it can be concluded that the conglomerate of HEMA and methyl cellulose was prepared successfully.

4.6 Biocompatibility:

Biocompatibility of hydrogels is determined by MTT assay. MTT solution added to hydrogels so that dehydrogenase enzymes of living cells contact with MTT and converted into purple color and those are not forming purple color then these hydrogels are containing dead cells or having toxicants so they remain in yellow color.



In this figure sample 6 showing purple color that means it more biocompatible to human body. So biocompatibility sample 6> sample 4>sample7> sample 1>sample 5>sample 2>sample 3.

CHAPTER 5 : CONCLUSION

The study reports of hydrogels of HEMA and methyl cellulose. The hydrogels were prepared as IPNs of HEMA and Methyl cellulose. Swelling behavior gave the drug delivery capacity of hydrogels, higher the conc. of Methyl cellulose better the swelling capacity. Where XRD gave crystalline property of IPN hydrogels. Mechanical study (tensile strength) having better results of samples .sample 4 i.e. 3:7(20/40) have better mechanical data, When hydrogels were subjected to repetitive mechanical stress. FTIR data of samples is showing better absorption property. Biocompatibility test given good number of active cells. These studies helps to enhance and correctives the properties of HEMA and Methyl cellulose hydrogels.

CHAPTER 5: REFERENCES

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